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Minireview

Recognition and selection of tRNA in translation

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Abstract Aminoacyl-tRNA (aa-tRNA) is delivered to the ribosome in a ternary complex with elongation factor Tu (EF-Tu) and GTP. The stepwise movement of aa-tRNA from EF-Tu into the ribosomal A site entails a number of intermediates. The ribosome recognizes aa-tRNA through shape discrimination of the codon–anticodon duplex and regulates the rates of GTP hydrolysis by EF-Tu and aa-tRNA accommodation in the A site by an induced fit mechanism. Recent results of kinetic measurements, ribosome crystallography, single molecule FRET measurements, and cryo-electron microscopy suggest the mechanism of tRNA recognition and selection.

Keywords: Translation; Rapid kinetics; Accuracy

1. Introduction

The ribosome is a large ribonucleoprotein complex that catalyzes the translation of a messenger RNA (mRNA) into protein. During elongation of the growing polypeptide, the mRNA codons are displayed one after another and aminoacyl-tRNAs (aa-tRNA) with complementary anticodons are selected. Elongation factor Tu (EF-Tu) delivers aa-tRNA to the ribosomal aa-tRNA binding site (A site) where tRNA recognition and selection takes place. Biochemical and kinetic studies have shown that the movement of aa-tRNA into the A site proceeds through a number of intermediate states, some of which could be isolated by using antibiotics or non-hydrolyzable GTP analogs [1–4]. Recently, significant progress in the characterization of these intermediates was achieved by single molecule fluorescence resonance energy transfer (smFRET) measurements [5]. Crystal structures showed how the decoding center specifically interacts with the codon and anticodon at each position of the duplex [6–8]. Finally, cryo-electron microscopy (cryo-EM) provided a wealth of information about the arrangements of aa-tRNA and EF-Tu on the ribosome and the contacts and conformational rearrangements of these molecules during decoding [9,10]. The goal of this review is to summarize these recent findings and to present a unifying mechanism of tRNA selection on the ribosome.

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2. Mechanism of aa-tRNA binding to the A site

Biochemical, kinetic, and smFRET studies have identified a number of intermediate states in the pathway of aa-tRNA delivery to the A site (Fig. 1) [1,5]. In the first step, the ternary complex forms a labile initial binding complex with the ribosome (rate constants of the forward and backward reaction are defined as k_1 and k_{-1}, respectively). Subsequent codon recognition (k_2, k_{-2}) triggers GTPase activation of EF-Tu (k_3), which is rate-limiting for GTP hydrolysis (k_{GTP}). Release of inorganic phosphate induces the conformational transition of EF-Tu from the GTP to the GDP form (k_4), whereby the factor loses the affinity for aa-tRNA and dissociates from the ribosome (k_6). The aa-tRNA released from EF-Tu is free to accommodate in the 50S A site (k_5) where it takes part in rapid peptide bond formation (k_{pep}). Alternatively, aa-tRNA may be rejected from the ribosome (k_7). The rate constants of the elemental steps of Fig. 1 were determined using the ternary complex containing Phe–tRNA^{Phe} and initiated ribosomes with UUU (cognate) or CUC (near-cognate) codons in the A site under conditions of high fidelity of aa-tRNA selection [11].

2.1. Initial binding

The initial step in the interaction of EF-Tu · GTP · aa-tRNA with the ribosome is codon-independent binding. The rate of binding is determined by EF-Tu interactions with the ribosome, because this step is much slower with aa-tRNA alone (unpublished data). At about 10^8 M^{-1} s^{-1} (20 °C) [5,12], the value for k_1 is unusually high, compared to typical second order rate constants of 10^5–10^7 M^{-1} s^{-1} that have been reported for other macromolecular systems. This indicates that the encounter of the ternary complex with the ribosome is not entirely random. One possible explanation is suggested by the observation that EF-Tu early in the sequence of A-site binding interacts with proteins L7/12 [13] of which four copies form the stalk of the 50S ribosomal subunit [14]. The formation of the initial binding complex may start with binding of EF-Tu to L7/12, most likely to one of the four highly mobile C-terminal domains, thus introducing a favorable statistical factor. Sequence and structure comparisons of the N-terminal domain of EF-Ts and the C-terminal domain (CTD) of L7/12 suggested the presence of a similar surface patch [15], indicating that helix D of EF-Tu may interact similarly with EF-Ts and with L7/12. In fact, mutational analysis suggested that helix D of EF-Tu and helices 4/5 of L7/12 are involved in the initial binding of the ternary complex to the ribosome. Based on mutagenesis and kinetic data, a structural model for the inter-

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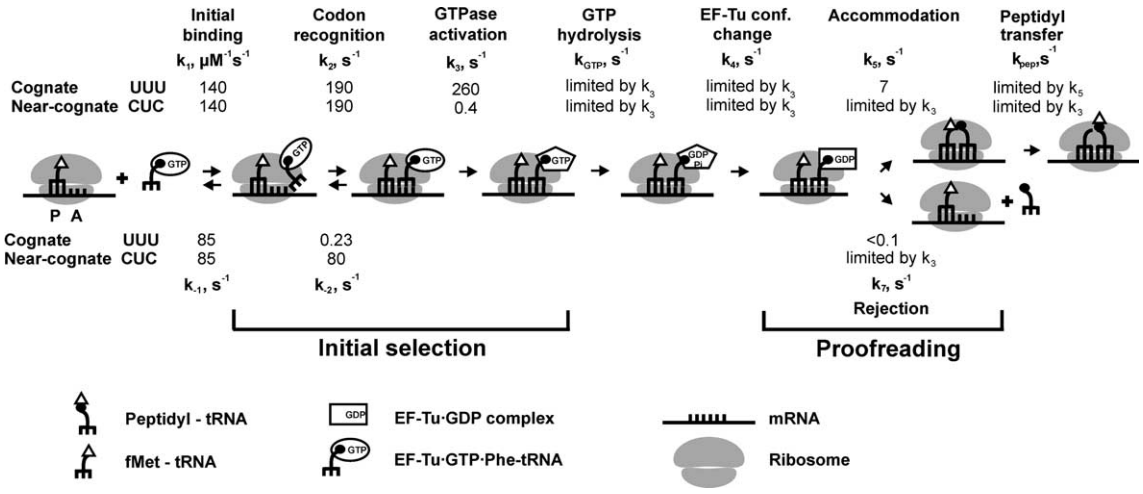

Fig. 1. Kinetic mechanism of EF-Tu-dependent aa-tRNA binding to the A site. Kinetically resolved steps are indicated by rate constants $k_1 - k_7$, k_{-1}, k_{-2} and the two chemical steps that are rate-limited by the preceding step are designated k_{GTP} and k_{pep}. Rate constants were determined at high fidelity conditions [11]. EF-Tu is depicted in different conformations in the GTP- and GDP-bound form and in the activated GTPase state. Dissociation of EF-Tu (k_6) (see text) is not shown for simplicity; it takes place concomitantly with, and independently of, aa-tRNA accommodation and rejection. The codon recognition step can be subdivided into (i) the codon reading by aa-tRNA (0.35 FRET; [5]) and (ii) the formation of the interactions of the ribosome with the codon–anticodon duplex [2,6] (0.5 FRET; [5]). The GTPase activation step can be distinguished from the codon recognition step by biochemical and stopped-flow kinetic experiments [11,17], but does not result in a smFRET change (0.5 FRET; [5]).

action of helix D of EF-Tu with helices 4 and 5 of L7/12 was proposed [13,15].

2.2. Codon recognition

Codon recognition step precedes through a number of intermediates identified by biochemical [16] and smFRET studies [5]. (i) Contact is established between anticodon of tRNA and the mRNA codon in the decoding center, imposing a fixed orientation on EF-Tu · GTP · aa-tRNA with respect to the P-site tRNA. This intermediate was recently observed by smFRET (0.35 FRET; [5]). (ii) The formation of the cognate codon–anticodon duplex leads to conformational changes of the conserved bases A1492, A1493, and G530 of 16S rRNA [6]. These bases change their positions and form A-minor interactions with the minor grove of the first two base pairs of the codon–anticodon complex in a fashion which is specific for Watson–Crick base pair geometry, but sequence independent. These rearrangements are induced or facilitated by correct codon–anticodon interaction; near-cognate aa-tRNA also induces some of these structural changes, but the resulting global interaction pattern is different (see below). The local structural changes at the decoding site constitute a part of the induced fit rearrangement of the ribosome.

The formation of the stable ribosome–codon–anticodon complex is reported by fluorescence changes in the anticodon region and the D loop of tRNA, and the complex can be stabilized by replacing GTP with the non-hydrolyzable GTP analog, GDPNP [16,17]; the A and P site tRNAs in this complex show 0.5 FRET [5] implying a further movement of aa-tRNA into the A site compared to the codon reading state (0.35 FRET). The formation of the stable complex is favored by purines at position 37 of tRNA and involves stronger stacking and binding of additional Mg^{2+} ions [18]. So far, the elemental rate constants of steps (i) and (ii) were not resolved. Kinetic analysis showed that the overall rate of codon recognition (k_2) is very similar for cognate and near-cognate ternary complexes [11,19]. However, a single mismatch at any position strongly destabilizes aa-tRNA on the ribosome (k_{-2}), almost independently of the nature or the position of the mismatch (unpublished data).

2.3. GTPase activation

GTPase activation of EF-Tu can be envisaged as a rearrangement of the active site that is necessary to assemble all groups involved in GTP hydrolysis in positions ready for catalysis; the exact structural mechanism of GTPase activation is not known. The interaction of cognate aa-tRNA with the ribosome increases the rate of GTPase activation [17], whereas mismatches in the codon–anticodon duplex impair the reaction [11,19] (Fig. 1). Local changes within the decoding site lead to a global change of the 30S subunit from an open to a closed conformation [7]. In the presence of a near-cognate aa-tRNA the closed conformation of the 30S subunit does not seem to form, as in crystals of the respective 30S complexes the shoulder of the subunit does not move and the head moves in a different direction [7]. Restricting the flexibility of the 30S subunit by the antibiotic streptomycin [8,20] affects the GTPase activation step primarily by decreasing the rate of the reaction with the cognate, and increasing the rate with the near-cognate ternary complex, resulting in an almost complete loss of selectivity [21]. During the GTPase activation step the conformational signal originating in the decoding site is communicated to the GTPase center on the 50S subunit, accelerating rearrangement steps that precede and limit the rate of GTP hydrolysis [1], which constitutes another part of the induced fit mechanism, in addition to the local structural changes at the decoding site.

Fluorescence measurements suggested that in the GTPase-activated state, the aa-tRNA adopts a more open conformation than in the preceding and following intermediate states; this conformation could be stabilized by binding the antibiotic kirromycin to EF-Tu [16]. It is very likely, therefore, that the structural rearrangements of aa-tRNA found in the kirromycin-stalled complex by cryo-EM [9,10] appear as early as during GTPase activation. The anticodon arm of tRNA on

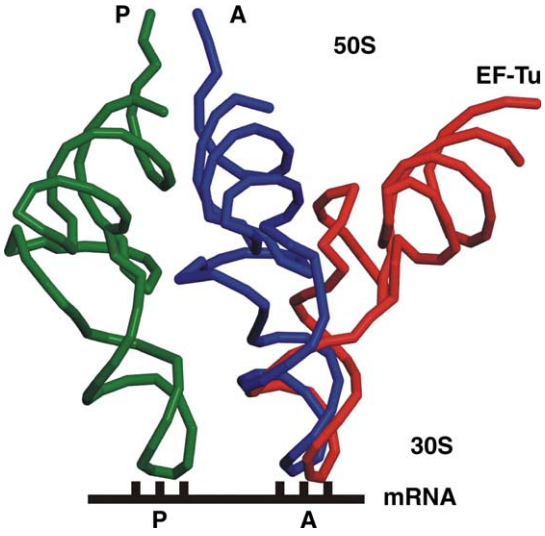

Fig. 2. Movement of aa-tRNA on the ribosome during A-site binding. tRNA in the P site (green) is taken from the crystal structure of *Thermus thermophilus* 70S ribosomes (1GIX; [32]); positions of aa-tRNA before (red) and after accommodation in the A site (blue) are from the cryo-EM model of the kirromycin-stalled ternary complex on the ribosome (1QZA; [10]) and from the 70S crystal structure [32], respectively.

the ribosome is reoriented by bending at the junction of the anticodon and D stems at nucleotides 44, 45, and 26 [10] (Fig. 2). The distorted structure of aa-tRNA is maintained by interactions between several elements of the ribosome, aa-tRNA, and EF-Tu [9,10]. Cognate codon–anticodon interaction and the global conformational changes of the ribosome may be required for the proper positioning of the ternary complex for GTPase activation and for bending of aa-tRNA.

Further support for the importance of local and global conformational changes at the decoding center comes from kinetic studies of the misreading inducing effect of the antibiotic paromomycin [21,22] combined with the crystal structures of 30S subunits with cognate or near-cognate substrates stabilized by the antibiotic [6,7]. Paromomycin is capable of inducing the same conformational change at the decoding center even in the absence of correct codon–anticodon interaction; formation of the complex stabilizes near-cognate aa-tRNA and increases the rate of GTP hydrolysis in the near-cognate ternary complex almost to the level of the cognate substrate.

On EF-Tu, the GTPase activation is likely to involve a rearrangement of the G domain of EF-Tu, which can be envisaged as a conformational change leading to opening of a hydrophobic gate that precludes the access of a catalytic His residue (see below) to the nucleotide binding pocket [23]. The rate enhancement of the GTPase activation can be achieved either by promoting the conformational rearrangements that correctly position the own catalytic groups of EF-Tu at the active site, or by donating additional catalytic groups in *trans* (see below). Residue Gly83 in EF-Tu plays an important role in both the rearrangement of the switch II region upon GTPase activation, due to the conformational flexibility inherent to Gly residues, and in GTP hydrolysis itself, probably by helping to position the catalytic water by hydrogen-bonding with the main chain oxygen of Gly83 [24] (Fig. 3).

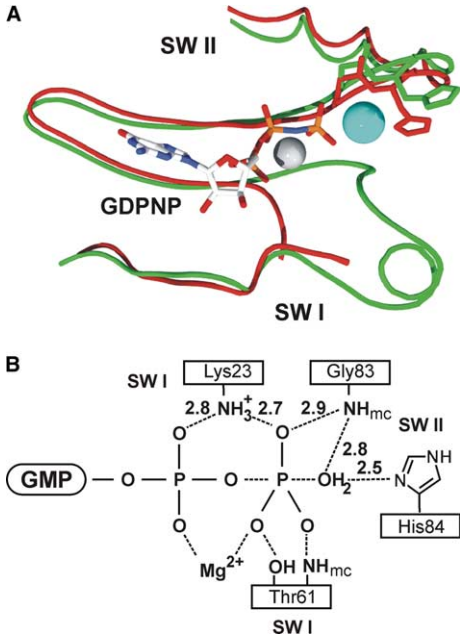

Fig. 3. Orientation of His84 in the active site of EF-Tu. (A) Positions of His84 in the crystal structures of EF-Tu · GDPNP (green, PDB 1EXM) and EF-Tu · GDP · aurodox (red, 1HA3) are shown relative to the hydrolytic water molecule (cyan), Mg^{2+} ion (gray) and GDPNP (1EXM). Structures were aligned on Lys23 of EF-Tu, which occupies the same position in the different structures. SWI and SWII, switch I and II regions. Part of the switch I region (amino acids 53–61) is not resolved in the EF-Tu · GDP · aurodox structure. (B) Model of the transition state of GTP hydrolysis. Interactions between residues important for catalysis and GTP are inferred from the superposition of the EF-Tu · GDPNP and EF-Tu · GDP · aurodox structures. The positions of Lys23, Gly83 and His84 are taken from the EF-Tu · GDP · aurodox complex, those of water, Mg^{2+}, GDPNP, and Thr61 from EF-Tu · GDPNP. The distance between N of His84 and O of γ-phosphate is 3–5 Å, depending on the His84 rotamer used.

2.4. GTP hydrolysis by EF-Tu on the ribosome

His84 in the switch II region of EF-Tu is directly involved in the chemistry step of the reaction, as the H84A mutation reduces the rate of GTP hydrolysis by 5 orders of magnitude, while all the steps preceding GTP cleavage are practically not affected by the mutation [25]. Furthermore, the rate of GTP hydrolysis is independent of pH in the range where the His side chain can ionize, suggesting that a general-base function of His84 in the reaction is unlikely [25]. Thus, the presumed catalytic role of His84 is to form hydrogen bond(s) to the substrates of the reaction, the attacking water molecule and/or the γ-phosphate, to precisely align groups directly involved in the reaction, in a similar way as Gln stabilizes the transition state of GTP hydrolysis in Ras-like or heterotrimeric G proteins [26] (Fig. 3). The intrinsic arginine residue (Arg58), that is located in the switch I region of EF-Tu in a position homologous to that of the catalytic arginine in G_α proteins, is not essential for GTP hydrolysis [27], and an extensive mutagenesis search did not identify any other side chains important for GTP hydrolysis in *Escherichia coli* EF-Tu [27–30].

Cryo-EM reconstructions showed extensive interactions of the switch regions of the G domain with the sarcin–ricin loop (SRL) of 23S rRNA [9,10], indicating that the SRL stabilizes the transition state conformation of the switch regions of the

factor. The smFRET measurements indicated that the cleavage of the SRL impedes progression from the GTPase activated state [5]; as a result, GTP hydrolysis by EF-Tu is abolished. Other contacts which potentially may contribute to GTP hydrolysis include ribosomal proteins L7/12, L11, and the L11-binding region of 23S rRNA [9,10]. The interaction of EF-Tu with L7/12 accounts for a 2500-fold stimulation of GTP hydrolysis [31], albeit none of the conserved side chains in the C-terminal domain of L7/12 was specifically responsible for the effect (unpublished data). However, all these contacts seem to take place far from the nucleotide binding pocket and therefore must act indirectly by inducing or stabilizing conformational transitions of EF-Tu, rather than by donating catalytic groups for GTP hydrolysis.

2.5. Pi release and the conformational change of EF-Tu

GTP hydrolysis yields the reaction products, GDP and inorganic phosphate, Pi, in the nucleotide binding site of EF-Tu. Direct measurements of the rate of Pi release indicated that it limits the rate of the conformational change of EF-Tu from the GTP- to the GDP-form (unpublished data). The release of aa-tRNA from EF-Tu probably takes place during the conformational transition, although it has not been observed directly. The rate of this step is likely to be determined by the flexibility of EF-Tu residues involved in the reorientation of the switch I and II regions required for the Pi release and the GTP/GDP switch [24].

2.6. aa-tRNA accommodation in the A site vs. rejection

In comparison to the crystal structures of ribosome-tRNA complexes [6,32], the cryo-EM structures [9,10] show that the anticodon arm of the A-site tRNA is positioned similarly in the codon-recognition and the accommodated states, in keeping with the similar footprints on ribosomal RNA of accommodated aa-tRNA and aa-tRNA in the ternary complex [33]. However, the 3′ end of aa-tRNA has to move a distance of almost 70 Å within the ribosome from its binding site on EF-Tu into the peptidyl transferase center (PTC), leading to the maximum smFRET (0.75; [5]) (Fig. 2). Aa-tRNA accommodation in the PTC limits the rate of peptide bond formation, which is intrinsically very rapid [19,34]. Accommodation of cognate aa-tRNA proceeds rapidly and efficiently, with essentially no drop-off of aa-tRNA at this step. In contrast, most of near-cognate aa-tRNA is rejected due to both low stability of aa-tRNA binding and lower rate of accommodation. This implies that the communication between the functional centers of the 30S and 50S subunits and induced fit are also important during proofreading (Fig. 1).

3. Fidelity of aa-tRNA selection

During translation of mRNA into protein by the ribosome, accurate selection of aa-tRNA depends upon the correct pairing of three bases between the mRNA codon and the tRNA anticodon. It is known that tRNA selection by the ribosome is significantly more accurate than expected from the thermodynamics of codon–anticodon pairing in solution. In the cell, concentrations of tRNAs specific for a particular amino acid, or for the same amino acid but a particular codon (isoacceptor tRNAs) varies between 1% and 10% of total tRNA. This implies that a large excess of incorrect ternary complexes competes with the single correct substrate for binding to the A site at a given codon. The fidelity of discrimination is very high, with only one incorrect per 1000–10 000 correct amino acids incorporated into protein [35]. Kinetic and mechanistic studies showed that the rejection of incorrect tRNAs occurs in two stages that are separated by irreversible GTP hydrolysis: initial selection of ternary complexes EF-Tu · GTP · aa-tRNA and proofreading of aa-tRNA [1,36,37]. The ribosome enhances the differences in the stabilities of different codon–anticodon complexes and specifically accelerates the rates of GTPase activation and accommodation of correct substrates, implicating duplex stability and induced fit as sources of selectivity [1,19,22].

3.1. Contributions of initial selection and proofreading

The relative contributions of initial selection and proofreading to overall selection in vivo are not known. In vitro kinetic and biochemical experiments indicated that during proofreading alone, about one amino acid out of 10–100 near-cognate aa-tRNAs was incorporated into peptide [11,19,36–38]. This suggested that in order to achieve the high overall selectivity, the initial selection step must be about as accurate as proofreading, i.e., not more than one out of 10–100 near-cognate ternary complexes should pass the initial selection screen. In fact, early estimations suggested that the efficiency of initial selection may indeed be that high [39,40], although it appeared to be strongly dependent on the experimental conditions, particularly the concentration of Mg^{2+} [19,41]. Recent kinetic experiments carried out at high fidelity conditions gave an incorporation of one incorrect per 450 correct amino acids, indicating an efficiency of initial selection of 30 [11]. Thus, both selection steps, initial selection prior to and proofreading after GTP hydrolysis, are required for efficient tRNA discrimination under any experimental conditions, and it is therefore very likely that the two steps operate also in vivo.

3.2. Kinetic versus thermodynamic discrimination

The important question is why the differences in the thermodynamic stabilities between the cognate and near-cognate aa-tRNA on the ribosome should not be sufficient for high-fidelity selection. In order to achieve high total selectivity, not more than one incorrect in 10–100 correct amino acids is allowed at each selection step. At equilibrium, this would require a ΔG^0 difference of about 1.3–2.7 kcal/mol in the thermodynamic stabilities of cognate and near-cognate aa-tRNAs. Indeed, the observed energetic penalty for a first-position C–A mismatch, 3.4 kcal/mol [11], would be sufficient to discriminate between correct and incorrect substrate, provided the binding step reached equilibrium. However, A-site binding is a non-equilibrium process that is driven by the rapid, irreversible forward reactions of GTP hydrolysis and peptide bond formation, such that the stability differences are not effective for discrimination. Rather, discrimination is based exclusively on the large differences (650-fold; [11]) in the forward reaction rates of GTPase activation and accommodation, which are high with correct and low with incorrect substrates and are sufficient to explain the observed levels of discrimination.

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